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14. ABSTRACT In this project, we proposed to use neural cells derived from human induced pluripotent stem cells (iPSCs) to establish MYC-driven medulloblastoma (MB) model. This model will be used to identify the gene network regulated by MYC. We will also study the interaction between MYC and Atoh1, another essential transcription factor for MB development. Our results support that neural progenitors and neural stem cells induced from human induced pluripotent stem cells can be efficiently transformed by MYC oncogene to form aggressive brain tumors that recapitulate human group 3 MB. We further show that it is feasible to establish neurosphere cultures from these MB tumors to enrich brain tumor stem cells. This neurosphere culture model are suitable for studying gene functions and also testing novel therapies. We further found that Atoh1 induction in this MYC-driven MB model significant promote tumor cell growth <i>in vitro</i> and tumor formation <i>in vivo</i> . We have also identified genome-wide MYC gene targets in this novel MB model and have further shown that Atoh1 modulates the transcriptional activity of MYC. Overall, this new MYC-driven MB model we established provides a reliable model for developing and testing potential therapies for this highly aggressive pediatric brain tumors. It is also feasible to use this MB model derived from human stem cells to perform personalized drug discovery and investigate the role of individual's distinctive genetic background in carcinogen sensitivity and MB susceptibility.					
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Introduction:

Brain tumors are the most common cause of childhood oncological death, and medulloblastoma (MB) is the most common malignant pediatric brain tumor. MB patients can be classified into four subgroups, and patients with Group 3 MB are more likely to have aggressive tumors and the worst prognosis. Group 3 MB is characterized by amplification and overexpression of MYC oncogene, herein referred to as MYC-driven MB. Modeling MYC-driven MB is critical for developing and testing potential therapies for this highly aggressive MB. Here, we will use neural cells derived from human induced pluripotent stem cells (iPSCs) to establish MYC-driven MB model. This model will be used to identify the gene network regulated by MYC. We will also study the interaction between MYC and Atoh1, another essential transcription factor for MB development. If successful, our proposed research will establish a new model of human MYC-driven MB, which can be immediately utilized to test existing drugs or develop drugs for patients with Group 3 MYC-driven MB. This MB model derived from human stem cells will provide a personalized tumor model for drug discovery and investigating the role of individual's distinctive genetic background in carcinogen sensitivity and MB susceptibility. By studying this human MYC-driven MB model, we will further provide novel insights into MYC-regulated gene network and the cross-talk between MYC-regulated and Atoh1-regulated gene networks. Understanding the molecular signaling that drives this aggressive tumor will lead to the identification of new therapeutic targets for developing effective treatment to cure this disease. We anticipate our proposed research will generate a preclinical MB tumor model in the short term and show long-term clinically-relevant impact on pediatric brain tumor research through in-depth study of this model.

Keywords:

brain tumors, medulloblastoma, pluripotent stem cells, MYC, transcription factors, high-throughput sequencing.

Accomplishments:

Specific Aim 1: To establish a human-iPSC-derived MYC-driven MB model and test the effects of Atoh1 expression on tumor initiation and growth in this MB model.

Major Task 1: Derive NPCs and NSCs from human iPSCs and check marker expression. (Month: 1-4)

We have generated Atoh1+NPCs and CD133+ NSCs from human iPSCs and applied multiple markers (e.g. CD133, Atoh1, TUJ1, Sox2, Nestin) to confirm the cell identity of these NPCs and NSCs.

Milestone achieved: These cells are ready for testing MYC-driven tumor formation in immunodeficient mice. **Please see details in Annual report for Year 1.**

Major Task 2: Compare the ability of human-iPSC-derived Atoh1+ NPCs and CD133+ NSCs to generate MYC-driven MB tumors in Mice.

Subtask 1: Obtain ACURO approval for animal use. (Month: 1-4)

Subtask 2: Perform cell infection and transplantation. (Month: 5-7)

Subtask 3: Monitor animal survival and collect tumors from animals. (Month: 8-11)

Subtask 4: Pathological analysis. (Month: 11-13)

We infected iPSC-derived Atoh1-induced NPCs with lentiviruses expressing a stabilized form of MYC (MYCT58A) and dominant-negative p53 (DNp53). Transgene expression has been validated by western blotting. We transplanted these lentivirus-infected NPCs into the cerebellum of immunodeficient NSG mice.

We monitored animal survival and found aggressive tumor growth in these mice (median survival=33).

We also collected tumors for pathological analysis. These MYC-driven tumors were comprised of poorly differentiated, medium to large size cells which showed nuclear molding, prominent nucleoli and numerous mitotic (Ki67+) and apoptotic (Cleaved-Caspase-3+) cells. These tumors also expressed early neuronal lineage marker (β -tubulin III). All these features closely mimic human Group 3 MB. **Please see details in Annual report for Year 1.**

We have successfully established neurosphere cultures from MYC-driven MB tumors from human Atoh1+ NPCs. These neurosphere cultures can be maintained in culture for >20 generations without losing their sphere-forming capability, an in vitro hallmark of stem cell self-renewal. These MYC-driven MB tumor derived neurospheres show expression of neuronal lineage marker (TUJ1), but not glial (GFAP) lineage marker. They express markers for brain tumor stem cells, such as CD133 (>20%), Nestin and Sox2. They also express markers for human group 3 MB (LGR5 and NPR3). **Please see details in Annual report for Year 1.**

Subtask 5: tumor gene expression profiling using microarray. (Month: 11-13)

We have extracted RNAs from tumor tissues derived from Atoh1+ NPCs with the transfection of MYC+DNp53 (n=2). These RNAs have been subjected to Affymetrics microarray analysis for gene expression profiling. Our biostatistician Dr. Hongkai Ji has analyzed these microarray data and compared them with human WNT, SHH, Group C and D MB subtypes.

We found that tumors from this iPSC-derived MYC-driven MB model clustered more closely with human Group C MB (Figure 1A and 1B), supporting that this model recapitulates human Group C MB. The tumors from two individual mice as biological replicates clustered closely together (Figure 1A and 1B). The distance between these two biological replicates is significantly shorter than the distance to other human MB samples ($p < 0.01$), supporting strong experimental reproducibility (Figure 1). Our biostatistician Dr. Hongkai Ji determined that two biological replicates were sufficient for testing our working hypothesis that this MB model recapitulates human Group C MB.

We have also established MYC-driven MB from iPSC-derived neural stem cells (NSCs). Animals with these tumors showed similar tumor latency (medium survival: 36 days) when compared to mice bearing tumors from iPSC-derived MYC-transformed NPCs (Figure 3A). In the gene expression profiling experiment, we found that NSC-derived MB samples ($n=2$) more closely cluster with Group C MB than other MB subtypes (Figure 3B). In pathological analysis, we found that NSC-derived MB also expressed well-established markers for Group C MB (e.g. c-MYC and NPR3) but not expressed markers for WNT and SHH subgroup MB (e.g. YAP) (Figure 3C).

Therefore, our results support that iPSC-derived NPCs and NSCs both can be transformed by c-MYC to form highly aggressive MB recapitulating human Group C MB.

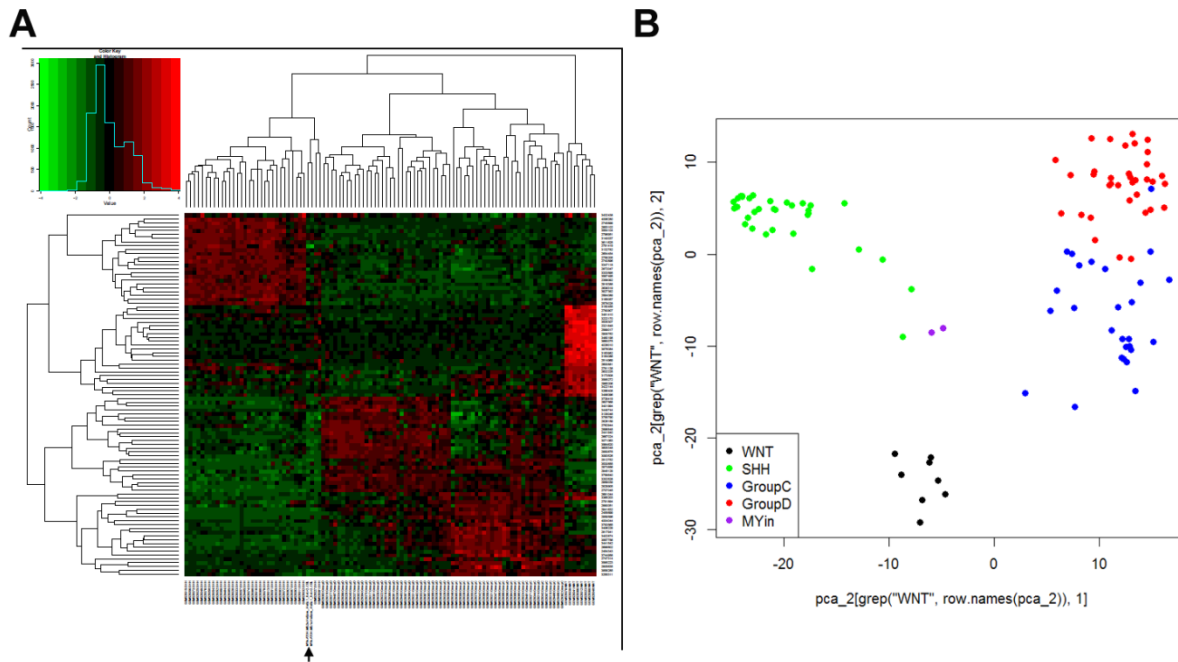


Figure 1. Gene expression profiling of the iPSC-derived MYC-driven MB model and clustering with human MB subtypes. RNAs were extracted from two tumor samples from the iPSC-derived MYC-driven MB model and subjected to CD133 microarray analysis. (A) Gene expression clustering was performed among these MB samples (arrow) and human MB samples from four MB subtypes. (B) Principle component analysis showed that these MB samples (purple dots) cluster with Group C MB samples.

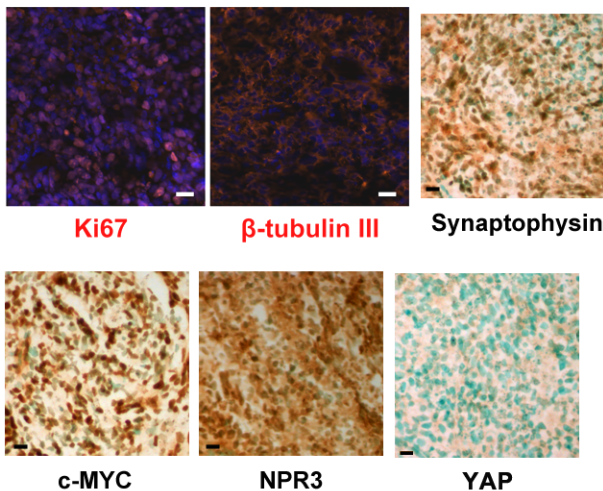


Figure 2. Histopathological analysis of the MYC-driven MB model derived from human NPCs differentiated from iPSCs. Tumor slices from the iPSC-derived MYC-driven MB model were subjected to immunohistochemistry (IHC) analysis for multiple MB and Group C MB markers. These tumors express MB markers (Ki67, beta-tubulin III and Synaptophysin) and Group C MB markers (c-MYC, NPR3), but do not express YAP, a marker positive for WNT and SHH subtype MB. (bar=50μm, nuclear counterstained with DAPI or methyl green)

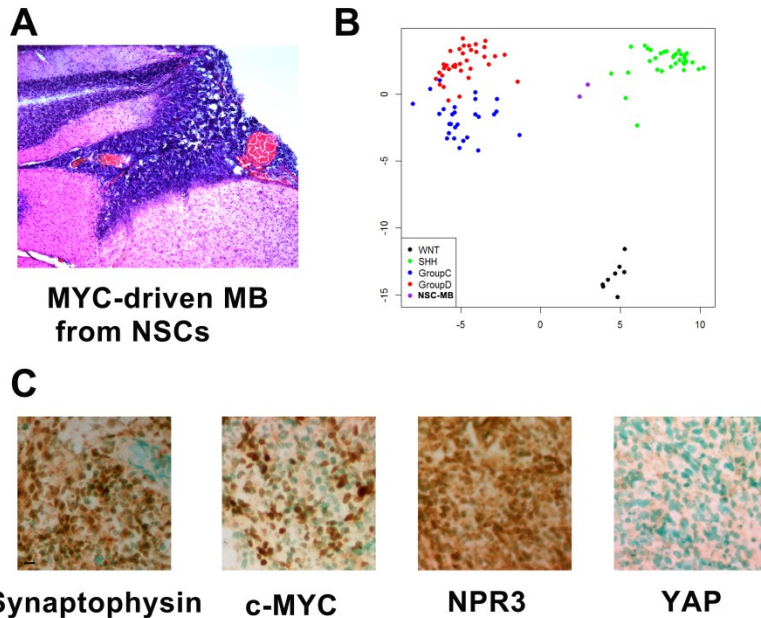


Figure 3 (Revision). MYC-driven MB model derived from human iPSC-derived NSCs. (A) MYC-driven MBs were established by transfecting iPSC-derived NSCs using MYC+DNp53. **(B)** mRNA samples from two tumors were subjected to microarray gene expression profiling. Principle component analysis showed that these MB samples (purple dots) more closely cluster with Group C MB. **(C)** Histopathological analysis showed that these tumors expressed MB marker Synaptophysin and Group C MB marker c-MYC and NPR3, but not SHH and WNT MB marker YAP. (bar=50μm, nuclear counterstained with DAPI or methyl green)

Subtask 6: Using microarray assay and pathological assay to compare MYC-driven MB tumors we generated with four human MB tumor subtypes and murine MYC-driven MB tumors (microarray data for human and murine MB tumors will be downloaded from the Gene Expression Omnibus database, pathological samples for human and murine MB tumors will be provided by Dr. Eberhart).

We have used microarray and histopathological analysis to compare the iPSC-derived MYC-driven MB model we established with human MB specimens. As shown in Figure 1B and 3B, tumor samples from NPC-derived and NSC-derived MB model were closely clustered with human Group C MB samples, supporting that these new models recapitulate human Group C MB. Microarray datasets from murine MYC-driven MB tumors were not used in this gene expression profiling analysis, because our biostatistician found that it is difficult to precisely convert mouse genes to human orthologs, and including murine MB models in our clustering analysis will not address our key hypothesis of this subtask that our MB models from human iPSCs recapitulate human Group C MB. Moreover, we also performed pathological analysis of tumor samples from our MB models and human Group C MB samples as the control. As shown in Figure 2 and Figure 3C, tumors from our new MB models express MB markers (Ki67, beta-tubulin III and Synaptophysin) and Group C MB markers (c-MYC, NPR3), but do not express YAP, a marker positive for WNT and SHH subtype MB. These results further support the results from gene expression profiling analysis.

Milestone achieved:

We have successfully established MYC-driven MB tumors from iPSC-derived NPCs and NSCs. We have also extensively characterized these tumors by using histopathological and molecular endpoints to confirm that these tumors recapitulate human group 3 MB. More importantly, we successfully established neurosphere cultures from these MYC-driven MB tumors. This cell model will greatly facilitate in vitro mechanistic studies and large-scale drug screen for targeting cancer stem cells in MYC-driven MB. We have used gene expression profiling and histopathological analysis to compare these novel MB models with various human MB subtypes. Our results support that we have successfully established iPSC-derived MB models for human Group C MB.

Overall, following the Statement of Work, we have achieved milestones of Subtask 1-6.

Major Task 3: Test the effects of enforced Atoh1 expression before and after MYC-driven MB tumors have formed.

Subtask 1: Perform cell transplantation in NOD/SCID mice (15 mice/each group, 4 groups, 60 mice total) (Month: 11-12).

Subtask 2: Induce Atoh1 expression in tumors, monitor animal survival and collect tumors (Month: 13-16)

Subtask 3: Pathological analysis of tumors with or without Atoh1 expression (Month: 17-19).

Subtask 4: Examine the expression of signature genes for MB subtypes (Month: 17-19).

Milestone achieved:

We have confirmed Atoh1 induction in neurospheres derived from MYC-driven MB tumors. Atoh1 transgene in these neurospheres can be induced by Doxycycline (Fig. 4A), thus allowing us to further study the effects of enforced Atoh1 expression before and after MYC-driven MB tumors have formed. We transplanted these cells into the brains of immunodeficient mice for MB tumor formation and Doxycycline treatment. Enforced Atoh1 expression promoted the growth of MB neurospheres (Fig. 4B and 4C). Enforced Atoh1 expression also significantly shortened the survival of tumor-bearing animals (Fig. 4D).

We have used 10 mice (n=5 for two groups) for survival analysis, 20 mice (n=10 each, two groups) for tumor size and pathological analysis, and 20 mice (n=10 each, two groups) for RNA and protein analysis. We further performed pathological analysis of tumors with or without Atoh1 expression. We found that Ki67+ cells in tumors with Atoh1 induction are significantly more abundant than tumors without Atoh1 induction ($62 \pm 8\%$ vs $38 \pm 9\%$, $p < 0.01$) (Figure 5). This result suggests that Atoh1 induction in this MB model promotes tumor cell proliferation. We are currently focusing on determine the underlying molecular mechanism. We are using the protein and mRNA samples from the harvested tumors for measuring the levels of cell cycle and apoptosis regulators.

We have also analyzed RNA and protein samples from tumors with or without Atoh1 induction. We did not find significant difference of the expression of markers for MB and Group C MB, including beta-tubulin III and Synaptophysin, c-MYC and NPR3 (data not shown).

Overall, following the Statement of Work, we have achieved milestones of Subtask 1-4.

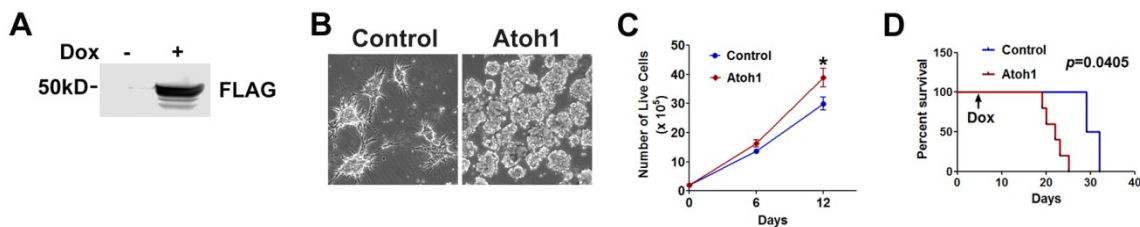


Figure 4. The effects of enforced Atoh1 expression on MB tumor growth. (A) MB neurospheres were treated with Dox for 24h, and protein samples were blotted with FLAG antibody to detect FLAG-tagged Atoh1. (B and C) Enforced Atoh1 in MB neurospheres promoted neurosphere growth. Bright field image of MB neurospheres with +/- enforced Atoh1 expression (B). Counting of live cells in MB neurospheres after 6 and 12 days of Dox-induced Atoh1 expression (C). (D) MB neurospheres were transplanted into the cerebellum of immunodeficient mice. Dox feeding through drinking water was initiated after 5 days. Enforced Atoh1 expression significantly shortened animal survival (n=5 in each group).

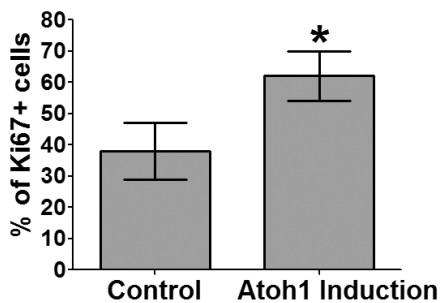


Figure 5 Atoh1 induction in MYC-driven MB promotes tumor cell proliferation. MB neurospheres were transplanted into the cerebellum of immunodeficient mice. Dox feeding through drinking water was initiated after 5 days. Enforced Atoh1 expression significantly increased the percentage of Ki67+ cells as determined by immunohistochemistry staining (n=10, $p < 0.01$).

Specific Aim 2: To identify the genome-wide MYC-regulated transcriptional network responsible for MYC-driven tumorigenesis, and to determine the effects of Atoh1 expression on MYC-regulated transcriptional events.

Subtask 1: perform MYC ChIP-seq in four different cell contexts (Months: 6-10)

- Subtask 2: perform RNA-seq in four different cell contexts (Months: 9-12)
- Subtask 3: Analyze ChIP-seq and RNA-seq dataset to identify gene targets directly regulated by MYC in different cell contexts; establish cell-context dependent and independent MYC target lists.
- Subtask 4: Perform pathway analysis on MYC target lists and determine the effects of Atoh1 expression on MYC-regulated transcriptional network.
- Subtask 5: Validate promoter binding and gene regulation in key signature genes regulated by MYC using ChIP-PCR and qRT-PCR.

In order to optimize the protocol for MYC ChIP suitable for our cell models, we have performed MYC ChIP on neurospheres isolated from MYC-driven MB tumors. Our initial experiments are not successful because we cannot collect enough cells for the ChIP experiment that requires large amount of cells. However, we have solved this problem by using neurosphere cultures from MYC-driven MB tumors that can be efficiently expanded in vitro (Fig. 3A). We have used MYC western blotting to confirm the immunoprecipitation efficiency of MYC ChIP, and we found that ChIP using MYC antibody efficiently precipitates MYC proteins from these neurospheres. To ensure the quality of our ChIP-seq samples, we have performed ChIP-PCR to confirm the binding of MYC to promoters of known MYC target genes, such as Prps1, Gart and Pfas (data not shown).

Progress achieved during the 3rd year with no-cost extension:

Aim 2 Subtask 1: We have prepared the MYC ChIP-seq libraries from two cell contexts for high-throughput sequencing. The ChIP-seq libraires have been sequenced by the JHMI High Throughput Sequencing Core and analyzed by our biostatistician Dr. Hongkai Ji and his student. As shown in Fig. 6, we analyzed the distribution of MYC binding sites, and we found that MYC mainly binds to distal intergenic regions and also binds to gene promoter and intron regions. The distribution of MYC binding sites is not altered by cellular contexts, when we compared the distribution of MYC binding sites between NPCs and NSCs (data not shown).

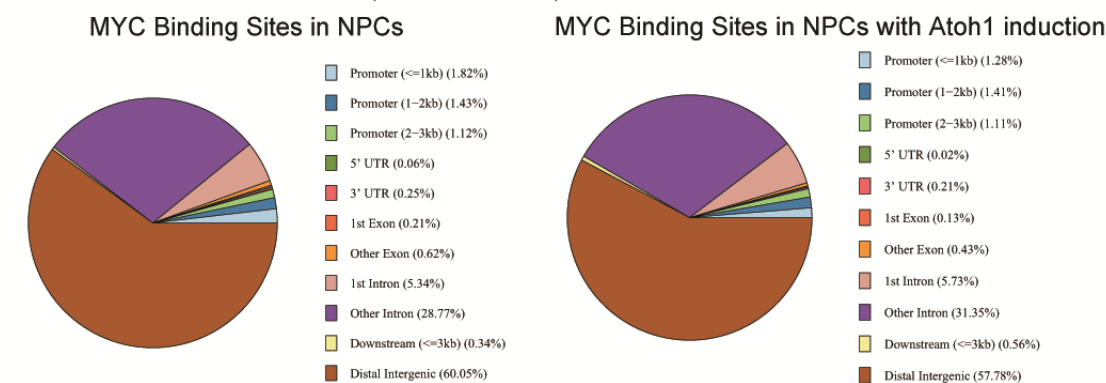


Fig. 6 Distribution of MYC binding sites in iPSC-derived NPCs with or without Atoh1 induction.

Aim 2 Subtask 2: We have also prepared RNA-seq libraries for identifying differentially expressed genes after MYC induction in both the NPC and NSC context. We have performed RT-PCR to ensure that know MYC-activated genes (e.g. Prps1, Gart and Pfas) are upregulated in response to MYC induction. We have sequenced the RNA-seq libraries from two cell contexts, and the RNA-seq datasets have been analyzed by our biostatistician to identify differentially expressed genes. Here, we show the clustering analysis results to support the reproducibility of the RNA-seq results between two experimental replicates (Fig. 7). Thus, these RNA-seq datasets are suitable to identify differentially expressed genes and signaling pathways as proposed in Subtask 3.

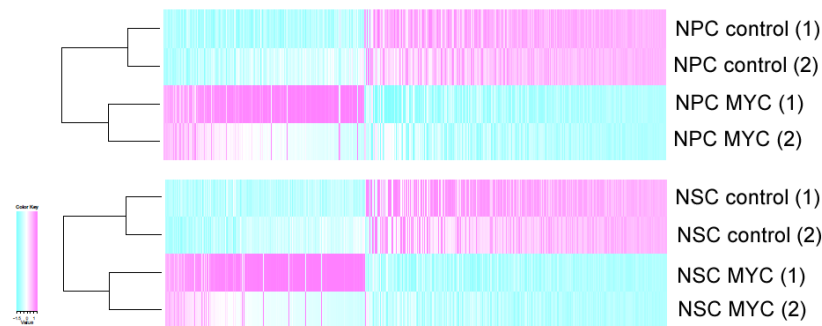


Fig. 7 Clustering of RNA-seq datasets in NPCs and NSCs with +/- Dox treatment for Atoh1 induciton (two replicates for each sample).

Aim 2 Subtask 3: We have annotated MYC binding sites as identified in ChIP-seq analysis to genes with at least one MYC binding site within 10 kb upstream and 5 kb downstream of their transcription start site (TSS) (referred to as a MYC bound gene). As shown in Fig. 8, in the context of NPCs, we identified 8,073 and 3,774 MYC-bound genes in cells with or without Atoh1 induction, respectively. 673 genes are overlap between these two lists. In the context of NSCs, we identified 13,180 and 5,963 MYC-bound genes in cells with or without Atoh1 induction, respectively. 1131 genes are overlap between these two lists. These results show for the first time that Atoh1 induction significantly alters the interaction between MYC and chromatin in both NPCs and NSCs.

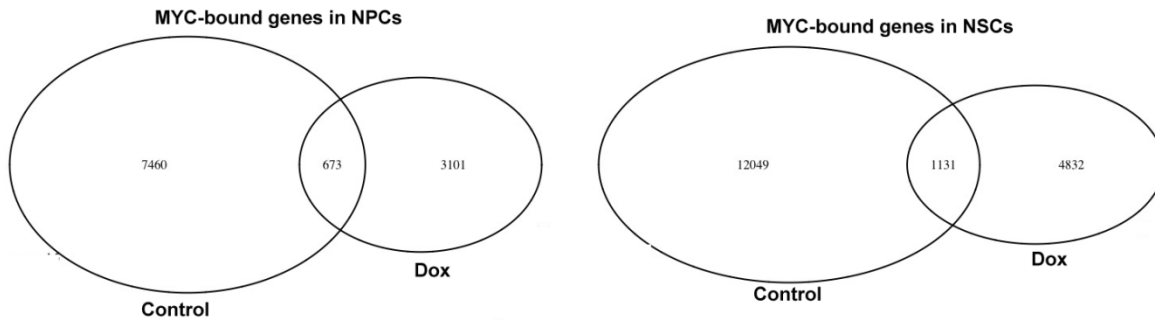


Fig. 8 MYC-bound genes as identified by ChIP-seq in NPCs and NSCs with +/- Atoh1 induction by Dox.

MYC bound genes have been further classified based on RNA-seq results into MYC-induced (fold change >2.0 , FDR <0.05) or MYC-repressed (fold change <0.5 , FDR <0.05) genes, which are defined as MYC-Up targets and MYC-Down targets, respectively. We have established gene lists for MYC-Up and MYC-Down targets in the context of NPCs and NSCs. In NPCs, there are 925 MYC-Up genes and 375 MYC-down genes. In NSCs, there are 1,433 MYC-Up genes and 575 MYC-down genes. We further establish the shared gene list through combining MYC-Up or MYC-down genes identified from NPCs and NSCs. There are 866 MYC-Up genes and 298 MYC-down genes that are identified as context-independent MYC target genes.

Aim 2 Subtask 4: We performed Ingenuity Pathway analysis of MYC-Up and MYC-down genes in cells with or without Atoh1 induction, in order to determine the effects of Atoh1 on MYC-regulated genes and signaling pathways. Fig. 9 shows the top 10 ranked MYC-regulated signaling pathways that are significantly altered by Atoh1 induction. We found that Atoh1 induction promotes the MYC-mediated activation of genes involved in Notch signaling and cell cycle regulation. This result is consistent with our previous finding (Fig. 4 and 5) showing that Atoh1 induction promotes cell proliferation in MYC-driven MB tumors and tumor-derived neurospheres. This result further suggests new hypothesis that targeting specific G-protein receptors (e.g. AT1R) as identified in the MYC-Up signaling list (G-Protein Coupled Receptor Signaling as shown in Fig. 9 MYC-Up genes) may inhibit Atoh1-induced tumor grow in MYC-driven MB. We are currently testing this hypothesis and have submit a R21 application to NIH for this project.

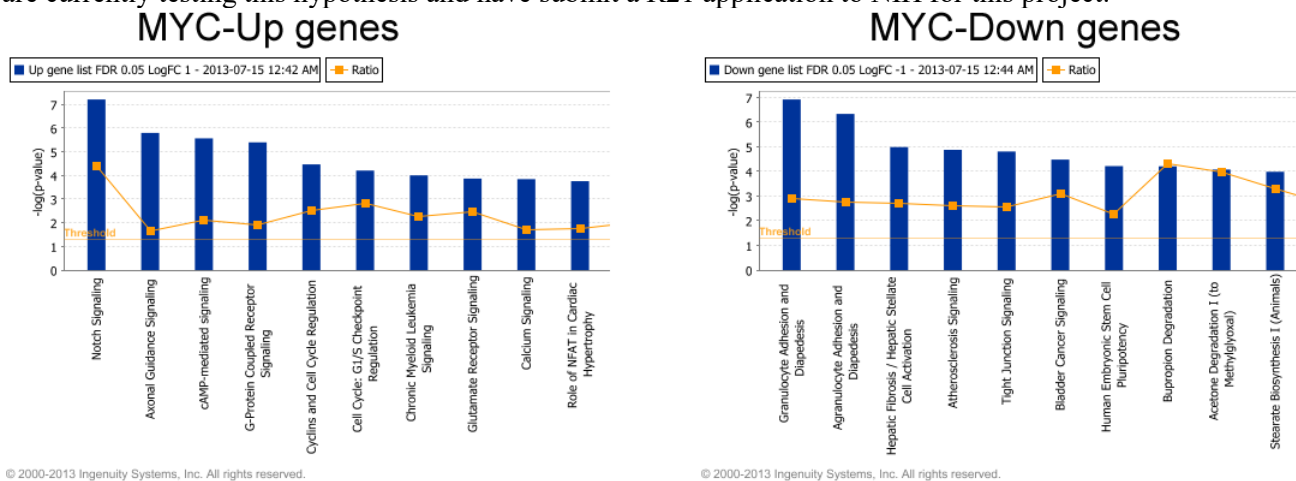


Fig. 9 Ingenuity pathway analysis of new MYC-up or MYC-down genes after Atoh1 induction.

Aim 2 Subtask 5: We have performed ChIP-PCR and qRT-PCR to validate our ChIP-seq and RNA-seq results. We have used primers to determine the expression of 70 key MYC-regulated genes after MYC enforced expression. We found that

62 of these genes identified by our RNA-seq results can be validated in qRT-PCR (Fig. 10 showing the results from 10 representative genes with essential roles in cancer cell proliferation and death).

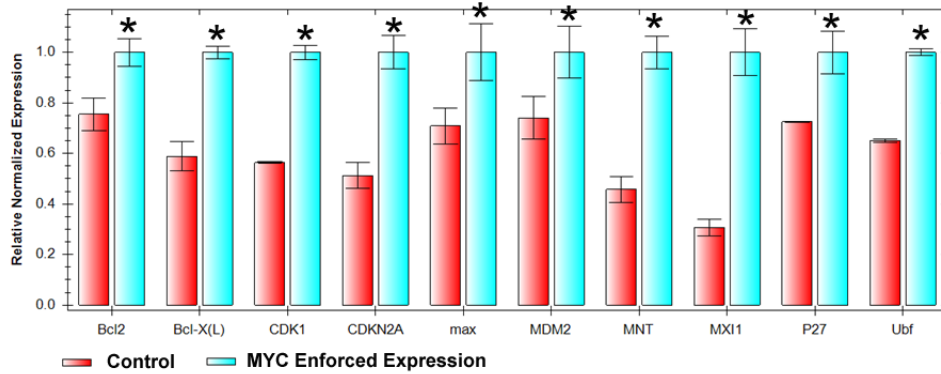


Figure 10. MYC gene targets were quantified by qRT-PCR in NPCs with or without MYC enforced expression (*: $p < 0.01$).

We have also used primers to determine the binding to MYC to the promoters of 30 genes. We are able to validate 24 genes as MYC-regulated genes. Fig. 11 shows the representative ChIP-PCR results of MYC binding in the promoter of 6 MYC target genes (APEX1, NDUFA3, RPL4, TSEM34, JUB and RPL-35) with two MYC unbound genes (ATP50 and POU5F1) as the negative control.

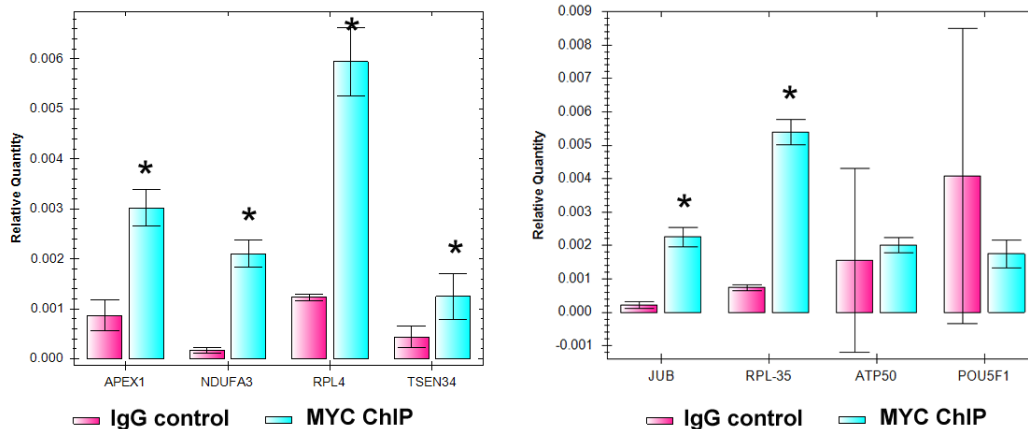


Fig. 11 NPCs with MYC enforced expression were subjected to MYC ChIP and qPCR to detect MYC binding sites in the promoters/enhancers of genes of interest as labelled (*: $p < 0.01$).
The promoters of ATP50 and POU5F1 serve as the negative control of MYC binding.

Overall, these qRT-PCR and ChIP-PCR results support the reproducibility of our ChIP-seq and RNA-seq datasets.

Milestone achieved during the 3rd year with no-cost extension:

During the 3rd year, we have achieved the milestone of all the subtasks as we proposed in Aim 2. We are currently focusing on two novel signaling pathways (the AngII-AT1R and PD-L1 signaling pathway) that may be co-regulated by MYC/Atoh1 and likely promote MB tumorigenesis. Following hypothesis-driven studies will likely identify novel MYC- and Atoh1-regulated oncogenic signaling pathways that can be targeted for inhibiting MB growth and immune escape. Currently, we will still need to perform in-depth bioinformatic analysis in combination with transcriptome data from MB patient database to identify more Atoh1-modulated MYC gene targets that will likely lead to new hypotheses and provide novel therapeutic targets for MYC-driven MB.

Training and Career Development Tasks:

Major Task 1: Training in analyzing MB tumors (Month: 1-12)

Subtask 1: Learn the principles of MB pathology

Subtask 2: Learn the pathology of four MB subtypes

Milestone achieved: These milestones have been achieved during the first year of this funding. **Please see details in Annual report for Year 1.**

Major Task 2: Career development in brain tumor research

Subtask 1: Attend a grant writing seminar

I have attend American Association for Cancer Research Annual Meeting 2015 and also American Brain Tumor Association Alumni Research Network Meeting 2014 and 2015. I have joined grant writing seminars during these meetings and improved my grant writing skill.

Subtask 2: Presentation at quarterly department brain tumor seminar

I have present my research in March 2014 at the Neuro-Oncology Research Meeting from Neurology department.

Subtask 3: Presentation at American Association for Cancer Research and Society for Neuro-Oncology annual meeting (2014 and 2015)

Milestone achieved: These milestones have been achieved. **Please also see details in Annual report for Year 1.**

Major Task 3: Organize results and prepare manuscript

Subtask 1: Organize results for one or two manuscripts

Subtask 2: Write manuscripts

Subtask 3: Design new hypothesis-driven projects and prepare preliminary data.

Subtask 4: Prepare one or two grant proposals.

Milestone achieved: We are currently preparing a manuscript focusing on this novel MYC-driven MB model and the following mechanistic studies. We anticipate submitting this paper in the next 3 months. In addition, we have used this novel MB model for testing novel therapeutic strategies. We found that targeting transcription factor TAZ effectively inhibit tumor cell growth *in vitro* and tumor formation *in vivo*. We also found that the stress hormone AngII is a MB tumor promoter and can be targeted for inhibiting MB tumor growth by repurposing FDA-approved drug Telmisartan. Building on these novel discoveries, we have submit two NIH R21 grant applications. One grant (1R21NS101400-01A1, Paracrine Angiotensin II Signaling Promotes Medulloblastoma through MYC Activation) has been scored with 15 percentile. The other R21 (1R21NS106407-01, Targeting TAZ-driven oncogenic signaling in Medulloblastoma) has been discussed in the NIH BMCT study section with a score of 4 percentile, and will likely be funded by NINDS (current fundable percentile of NINDS is 12 percentile).

How were the results disseminated to communities of interest?

Nothing to report.

Plan for the next reporting period:

None.

Impact:

Impact on the development of the principal discipline of the project:

The principal discipline of this project is to model MYC-driven medulloblastoma by using human stem cells and to further study the transcriptional mechanisms underlying the tumor formation process of MYC-driven medulloblastoma. Our current results strongly support that neural progenitors induced from human induced pluripotent stem cells by Atoh1 induction can be efficiently transformed by MYC oncogene to form aggressive brain tumors that recapitulate human group 3 medulloblastoma. We further show that it is feasible to establish neurosphere cultures from these medulloblastoma tumors to enrich brain tumor stem cells. This neurosphere culture model are suitable for studying gene functions and also testing novel therapies. We further found that Atoh1 induction in this MYC-driven MB model significant promote tumor cell growth *in vitro* and tumor formation *in vivo*. Overall, this new MYC-driven medulloblastoma model we established provides a reliable model for developing and testing potential therapies for this highly aggressive pediatric brain tumors. It is also feasible to use this medulloblastoma model derived from human stem cells to perform personalized drug discovery and investigate the role of individual's distinctive genetic background in carcinogen sensitivity and medulloblastoma susceptibility.

Impact on other disciplines: nothing to report.

Impact on technology transfer: nothing to report.

Impact on society beyond science and technology: nothing to report.

Changes/Problems:

We encountered a minor problem when we performed Subtask 1 of Specific Aim 2. Our initial experiments are not successful because we cannot collect enough cells for the ChIP experiment that requires large amount of cells. However, we have solved this problem by using neurosphere cultures from MYC-driven MB tumors that can be efficiently expanded in vitro. Using this alternative strategy, we have achieved the milestones as proposed in Aim 2 during the 12-month no-cost-extension period.

Products:Conference presentation:

A MYC-driven medulloblastoma model derived from human induced pluripotent stem cells.

Jonathan Sagal¹, Charles G. Eberhart², Mingyao Ying¹.

¹Kennedy Krieger Research Institute, Baltimore, MD;

²Johns Hopkins University School of Medicine, Baltimore, MD

Oral presentation in Mouse Models of Human Cancer 2 mini-symposium session.

2015 American Association for Cancer Research Annual Meeting

Monday, Apr 20, 2015

NIH R21 application:

Targeting TAZ-driven MYC Expression in Medulloblastoma (1R21NS106407-01)

Paracrine Angiotensin II Signaling Promotes Medulloblastoma through MYC Activation (1R21NS101400-01A1)

Participants & Other Collaborating Organizations:

Name: Mingyao Ying

Project Role: Principle investigator

Nearest person month worked: 4.8

Contribution to Project: Dr. Ying is responsible for supervising and finishing all the proposed experiments, and analyzing data and preparing publication.

Funding Support: NIH

Name: Charles Eberhart

Project Role: Designated Collaborator

Nearest person month worked: 0.6

Contribution to Project: Dr. Eberhart serve as Dr. Ying's mentor in the project and perform pathological analyses of medulloblastoma tumors.

Name: Hongkai Ji

Project Role: Collaborator

Nearest person month worked: 1.2

Contribution to Project: Dr. Hongkai Ji performed biostatistical analysis of our ChIP-seq and RNA-seq data.

APPENDICES

1. Summary Statement and specific aims of R21 grant reviewed by NIH
2. Specific aims of R21 with a score of 4 percentile
3. Abstract picked by AACR annual meeting for presentation
4. Biosketch of Mingyao Ying

SUMMARY STATEMENT

PROGRAM CONTACT:
JANE FOUNTAIN
301-496-1431
fountai@ninds.nih.gov

(Privileged Communication)

Release Date: 07/10/2017
Revised Date:

Application Number: 1 R21 NS101400-01A1

Principal Investigator

YING, MINGYAO

Applicant Organization: HUGO W. MOSER RES INST KENNEDY KRIEGER

Review Group: TME
Tumor Microenvironment Study Section

Meeting Date: 06/15/2017
Council: OCT 2017
Requested Start: 09/01/2017

RFA/PA: PA16-161
PCC: FOUNTJNE

Project Title: Paracrine Angiotensin II Signaling Promotes Medulloblastoma through MYC Activation
SRG Action: Impact Score:34 Percentile:15 +
Next Steps: Visit https://grants.nih.gov/grants/next_steps.htm
Human Subjects: 10-No human subjects involved
Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

Project Year	Direct Costs Requested	Estimated Total Cost
1	150,000	242,589
2	125,000	202,157
TOTAL	275,000	444,746

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R21NS101400-01A1 Ying, Mingyao**BIOHAZARD COMMENT**

RESUME AND SUMMARY OF DISCUSSION: This application proposes a novel hypothesis that endothelial cell (EC)-derived angiotensin II (AngII) interacts with AngII receptor (AT1R) on medulloblastoma (MB) cells which leads to induction of Myc expression to promote tumor growth and Myc in turn induces expression of AT1R creating a positive feedback loop and development of aggression tumor phenotype. The therapeutic utility of AT1R blockers for MB will also be assessed. The proposed studies have considerable significance as MB is a malignant pediatric tumor and there is some strong scientific premise to support the hypothesis, if successful, the findings may provide new insight on the mechanisms underlying the interaction between stromal ECs and tumor cells in tumorigenesis and therapeutic translation of repurposing FDA-approved AT1R blockers for treatment of MB, such as Group 3 MB with amplified Myc. The applicant has made a concerted effort to address the previous critiques to improve the application by adding literature references and providing data to support the hypothesis and revised the experimental plan to enhance scientific rigor. Major strengths are the relevant expertise of the applicant and his collaborating team, the novel paracrine positive feedback loop in EC-tumor cell interaction, some novelty on the repurposing of drugs for MB, rather straightforward experimental plan, and the resources availability to support the proposed studies. However, some weaknesses were also discussed. The transcription regulation of Myc by AngII has been reported long time ago and the application of AT1R blockers in other cancers has been reported, which somewhat weaken innovation and significance aspects of the project. Also, there are numerous concerns with some of the experimental approaches, which may diminish potential impact of the studies. The evidence presented in the application is not highly convincing to support the correlation to Myc expression in the proposed model. The off-target effects of the drugs have not been taken into consideration or controlled for in the experiments. The biological variables of sex/gender have not been properly addressed as the 2:1 (male:female) incidence ratio in Group 3 MB was overlooked and not considered. After thorough discussion, Reviewer 1 remained highly enthusiastic whereas the rest of the panel weighed in the perceived weaknesses and considered this a very good to excellent application. Consequently, this application falls within the outstanding merit ranking range. The proposed studies would likely have some high impact on the understanding of the contribution of the AngII, AT1R and Myc axis in modulation of EC-tumor cell interaction in medulloblastoma.

DESCRIPTION (provided by applicant): Medulloblastoma (MB) is the most common malignant pediatric brain tumor, and results in significant neurological, intellectual and physical disability or death. Group 3 MB, the most aggressive subtype, is frequently associated with MYC gene amplification and/or protein overexpression, hereby referred to as MYC-driven MB. The molecular mechanisms that drive MYC hyper-activation in MB remain incompletely understood. MB cells in actual tumors interact extensively with stromal cells. However, it is largely unknown how stroma-derived signals promote MYC expression and MB growth. This represents a substantial knowledge gap and hinders the development of effective MB therapies. This project focuses on the peptide hormone Angiotensin II (AngII), a mediator of the oncogenic interactions between MB cells and endothelial cells (ECs). Our preliminary studies identified a novel paracrine signaling mechanism in MB, where EC-released AngII induces MYC expression in MB cells and promotes MB cell growth. We further found that the MB-promoting effects of AngII are inhibited by silencing the AngII receptor AT1R and the FDA-approved AT1R blocker (ARB) Telmisartan, supporting the feasibility to use ARBs for clinical targeting of the AngII signaling in MB. To understand the molecular mechanisms, we identified a novel AT1R-MYC positive feedback loop in MB cells whereby AngII induces MYC expression through AT1R, and MYC induces AT1R expression to form a positive feedback loop. Overall, these preliminary data support our central hypothesis that AngII mediates oncogenic tumor-endothelial-cell interactions that promote MB cell tumorigenicity by activating a AT1R-MYC positive feedback loop. This project will more comprehensively study the functions and molecular mechanisms of this novel AngII-AT1R-MYC signaling pathway in MB. We will also study the systemic treatment of ARBs that cross the blood-brain barrier in pre-clinical MB models.

Paracrine Angiotensin II Signaling Promotes Medulloblastoma through MYC Activation

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, and results in significant neurological, intellectual and physical disability or death. Recent gene expression studies have identified four MB subtypes (WNT, SHH, Group 3 and 4) (1, 2). Group 3 MB, the most aggressive subtype, is frequently associated with MYC (c-Myc) gene amplification and/or protein overexpression, hereby referred to as MYC-driven MB. The molecular mechanisms that drive MYC hyper-activation in MB remain incompletely understood. A significant subset of MYC-high MBs do not harbor MYC genomic amplification or mutation, suggesting that other non-genetic mechanisms may also cause MYC deregulation. MB cells in actual tumors interact extensively with stromal cells (e.g. endothelial cells (ECs), normal brain cells and inflammatory cells). However, it is largely unknown how stroma-derived paracrine signaling regulates MYC expression and MB growth. This represents a substantial knowledge gap and hinders the development of effective MB therapies.

This project focuses on the peptide hormone Angiotensin II (AngII), a potential mediator of the oncogenic interactions between MB cells and stromal cells. Our initial studies identified a novel paracrine signaling mechanism in MB, where EC-released AngII induces MYC expression in MB cells and promote MB cell growth. From various MB and stromal cells we tested, only ECs express two Angiotensin-converting enzymes (Renin and ACE) and are capable to release AngII. MB cells express the AngII receptor AT1R, suggesting potential MB-EC interactions mediated by AngII. To support the functional significance of this tumor-stroma signaling pathway, we showed that synthetic and EC-released AngII promotes MB cell proliferation and induces the expression of MYC, a major oncogenic driver in MB. More importantly, the MB-promoting effects of AngII can be inhibited by silencing the AngII receptor AT1R and Telmisartan, an FDA-approved AT1R blocker (ARB) currently used for hypertension treatment, thus allowing the clinical targeting of the AngII signaling in MB. To further understand the molecular mechanisms, our results suggest a novel AT1R-MYC positive feedback loop in MB cells whereby AngII induces MYC expression through AT1R, and MYC induces AT1R expression to form a positive feedback loop. These preliminary results support a novel AngII-AT1R-MYC signaling pathway in MB that is likely activated by MYC deregulation and further enhances MYC expression when MB cells in actual tumors are exposed to EC-released AngII. Now, it is necessary to more comprehensively and rigorously determine the cellular effects and molecular mechanisms of this AngII-AT1R-MYC signaling pathway in MB. It is also feasible to target this pathway for MB treatment by using ARBs (e.g. Telmisartan) that cross the blood-brain barrier (BBB).

General Hypothesis: The peptide hormone AngII mediates oncogenic tumor-endothelial-cell interactions that promote MB cell tumorigenicity by activating a AT1R-MYC positive feedback loop.

Our goals are (1) to define a novel paracrine signaling mechanism that induces MYC expression and promotes MB cell tumorigenicity, and (2) to establish a clinically translatable strategy for MB treatment based on repurposing ARBs.

Specific Aims:

Aim 1. Determine if AngII promotes MB cell tumorigenicity through a AT1R-MYC positive feedback loop.

This aim will build on our working model that AngII mediates oncogenic tumor-endothelial-cell interactions in MB. We will use a panel of MB cell lines and a novel MYC-driven MB model derived from human induced pluripotent stem cells. **Subaim 1A** will comprehensively study the effects of AngII on MB cell growth, migration, invasion and tumor propagation, and further determine if AT1R and MYC mediate these effects by using MB cells with AT1R and MYC silencing. **Subaim 1B** will extensively study the signal transduction mechanism of the AngII-AT1R-MYC signaling pathway, and also determine if AngII induces MYC through transcriptional and/or post-translational mechanisms. Positive results will uncover a novel stromal-cell-induced molecular mechanism that can be targeted for MYC inhibition and MB treatment.

Aim 2: Repurpose AT1R blockers to inhibit MB growth and suppress MYC expression in tumors.

Our preliminary data demonstrate that ARBs inhibit AngII-induced MYC activation and AngII-induced MB cell growth. Certain ARBs (e.g. Telmisartan and Losartan) cross the blood-brain barrier, supporting the feasibility of systemic ARB treatment for MB therapy. **Subaim 2A** will determine if systemic treatment using two ARBs (Telmisartan and Losartan) inhibits MB xenograft growth and MYC expression in tumors. **Subaim 2B** will use a novel species-specific transcriptome analysis to globally identify ARB-regulated pathways in human tumor cells and mouse stromal cells in xenografts. We will determine if ARBs inhibit MYC target gene signature and other MB-promoting signaling pathways in tumor cells. Transcriptome analysis of stromal cells will likely provide novel insights into ARB's effects on tumor angiogenesis and inflammation. Overall, positive results will lay a solid foundation for repurposing ARBs for MB therapy.

Status Information ?

Contacts

Administration: Scientific Review Administrator(SRO))
Name: Rahman Sesay, Lambratu
Phone: 301-905-8294
Email: rahmanl@mail.nih.gov
Administration: Grants Management Specialist(GMS)
Name: Mayer, Pamela L
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Email: mayerp@ninds.nih.gov
Administration: Program Official(PO)
Name: FOUNTAIN, JANE W.
Phone: 301-496-1431
Email: fountai@ninds.nih.gov

Grant Number: 1 R21 NS106407-01

Status

Status: Scientific Review Group review completed: Council review pending. Refer any questions to Program Official.

Last Status Update Date: 09/21/2017
PI Name: Ying, Mingyao

Institution Name: HUGO W. MOSER RES INST KENNEDY KRIEGER

NIH Appl. ID: 9508849

School Name:

School Category:

Division Name:

Department Name:

Proposal Title: Targeting TAZ-driven oncogenic signaling in Medulloblastoma

Proposal Receipt Date:

06/15/2017

Project Period Begin Date:

04/01/2018

Project Period End Date:

03/31/2020

Application Source: Grants.gov

Current Award Notice Date:

eApplication Status: Submission Complete

FOA: [PA16-161] - NIH EXPLORATORY/DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21)

Other Relevant Documents

[e-Application](#)

[eSubmission Cover Letter](#)

[eSubmission-PHS Assignment Request Form](#)

Additions for Review

Review

Application

Study Section

Advisory Council (AC)

Award Document Number:

RNS106407A

FSR Accepted Code: N

Snap Indicator Code:

Impact Score: 24

Percentile: 4.0

For information about next

steps: Click [here](#)

Early Stage Investigator Eligible:

New Investigator Eligible:

Eligible for FFATA Reporting: Yes

Scientific Review Group: BMCT

Council Meeting Date

(YYYY/MM): 2018/01

Meeting Date: 09/18/2017

Meeting Time: 08:00

Study Roster: [View Meeting](#)

[Roster](#)

Institute/Center Assignment

Institute or Center	Assignment Date
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE (Primary)	06/15/2017
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE (Primary)	06/28/2017

Status History

Effect Date	Status Message
07/03/2017	Scientific Review Group review pending. Refer any questions to the Scientific Review Administrator.
06/15/2017	Application entered into system

Reference Letter(s)

This list shows Reference Letters associated with this particular Grant Application. Principal Investigator can see a list of all Reference Letters within Personal Profile - Reference Letters section on eRA Commons

Targeting TAZ-driven MYC Expression in Medulloblastoma

Background:

Medulloblastoma (MB), the most common malignant pediatric brain tumor, results in significant neurological, intellectual and physical disability or death. Four MB subtypes (WNT, SHH, Group 3 and 4) have recently been identified (1-3). Group 3 MB, the most aggressive subtype, is more frequently associated with gene amplification and/or protein overexpression of the MYC (c-Myc) oncogene, hereby referred to as MYC-driven MBs. It is still unclear how MB cells activate and maintain high MYC expression, as only a small portion of MYC-high MBs is caused by MYC genomic amplification. MYC-targeted MB therapies are also lacking.

Here, we propose to study the transcription co-activator TAZ, a novel MYC upstream activator and a potential therapeutic target for inhibiting MYC-driven MBs. Targeting MYC activators is a promising strategy for inhibiting MYC-driven MBs, when compared to direct MYC inhibition that is still challenging. For example, the small molecule JQ1 inhibits the BET-family bromodomain protein BRD4, a well-studied MYC activator, and also inhibits MYC-driven MBs in pre-clinical testing (4, 5). However, blocking a single MYC activator in patients may show limited therapeutic efficacy and will likely lead to therapeutic resistance, as MYC expression in heterogeneous human tumors is often driven by a complex molecular network containing multiple collaborative and compensatory regulators (6). Having the capability to target multiple MYC activators will allow us to more effectively inhibit MYC-driven MBs by combination therapy, and will also provide secondary therapy when primary MYC-targeted therapy fails.

Currently, key MYC activators in MBs are still incompletely understood. Here, we propose to fill in this knowledge gap by defining TAZ as an essential and druggable MYC activator for treating MYC-driven MBs.

Preliminary Studies:

We found for the first time that TAZ is an essential MYC activator in MB cells. TAZ but not its paralog YAP is highly expressed in MYC-amplified MB cells and their xenografts. TAZ silencing downregulates MYC and inhibits MB cell proliferation in vitro. Enforced TAZ expression induces MYC expression in MB cells. We further showed that TAZ forms complex with transcription activator TEAD to bind to MYC enhancers. For pharmacological TAZ targeting, we showed that the FDA-approved drug Verteporfin (VP) inhibits TAZ-TEAD interaction, suppresses MYC expression and inhibits MB cell tumor propagation.

Overall, our preliminary results strongly support that TAZ is a MYC activator in MB cells and a potential target for MYC inhibition, and also support that VP can be repurposed to treat MYC-driven MBs.

General Hypothesis: The TAZ-TEAD transcription complex activates MYC expression in MB cells through binding to and transactivating MYC enhancers. Targeting TAZ suppresses MYC expression and inhibits the tumorigenicity of MYC-driven MB cells.

Our goals are (1) to uncover a novel TAZ-driven mechanism that activates MYC expression and promotes MB cell tumorigenicity, and (2) to further establish a clinically translatable strategy for MB treatment based on repurposing the FDA-approved drug VP for targeting TAZ and inhibiting MYC-driven MBs.

Specific Aims:

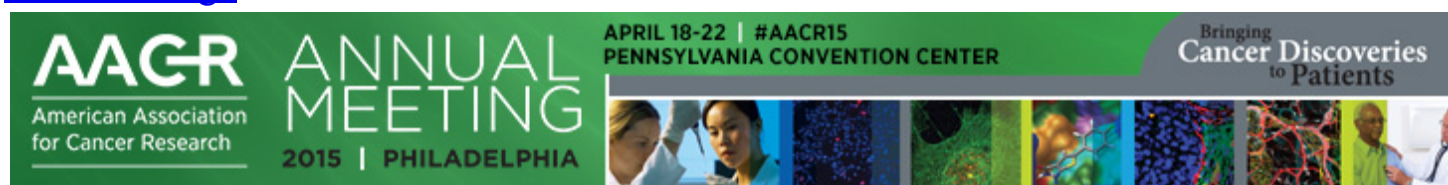
Aim 1. Determine if TAZ promotes MB cell tumorigenicity through transcriptional activation of MYC.

This aim will use patient-derived MB cell lines as well as a novel MYC-driven MB model we recently developed by using human neuronal precursor cells (NPCs) derived from induced pluripotent stem cells (iPSCs). We will determine: (1) if TAZ silencing in MYC-high MB cells inhibits MYC expression and tumor propagation, (2) if enforced TAZ expression activates MYC expression and induces tumor formation from MYC-low/absent non-tumorigenic MB cells and normal NPCs, and (3) if the molecular mechanism underlying TAZ-driven MYC activation involves MYC transactivation by the TAZ-TEAD complex. If successful, we will provide novel mechanistic insights into MYC activation in MB cells, and will clearly define TAZ as a therapeutic target for inhibiting MYC-driven MBs.

Aim 2. Determine if the FDA-approved drug Verteporfin inhibits MYC-driven MB in vivo, and further determine if Verteporfin inhibits TAZ-driven MYC activation.

This aim is based on our working model that VP directly binds to TAZ and inhibits MYC activation by the TAZ-TEAD complex. We will perform the first drug efficacy study of VP on a panel of MB cell cultures and xenografts. By using novel VP-loaded nanoparticles, we will determine for the first time if systemic VP treatment suppresses TAZ and MYC expression in MB xenografts and inhibits tumor propagation. We will further study the molecular mechanism of VP's drug action based on our working model that VP inhibits MB cells through MYC inhibition by blocking the interaction between the TAZ-TEAD complex and MYC enhancers. The success of these studies will lay a solid foundation for repurposing VP for treating high-risk MYC-driven MBs and likely other MYC-driven human cancers.

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Presentation Abstract

Abstract
Number: 2892

Presentation Title: A MYC-driven medulloblastoma model derived from human induced pluripotent stem cells

Presentation Time: Monday, Apr 20, 2015, 3:35 PM - 3:50 PM

Location: Room 121, Pennsylvania Convention Center

Author Block: Jonathan Sagal¹, Charles G. Eberhart², Mingyao Ying¹. ¹Kennedy Krieger Research Institute, Baltimore, MD; ²Johns Hopkins University School of Medicine, Baltimore, MD

Abstract Body: Brain tumors are the most common cause of childhood oncological death, and medulloblastoma (MB) is the most common malignant pediatric brain tumor. Current MB treatments yield five-year survival rates of 60-70%, but usually result in significant neurological, intellectual and physical disabilities. Recent gene expression studies have identified four MB subgroups, many of which have unique clinical and histopathological features. Patients with Group 3 MB are more likely to have aggressive and invasive tumors with large cell/anaplastic histology, and have the worst prognosis. Group 3 MB is characterized by amplification and overexpression oncogenic transcription factor MYC, herein referred to as MYC-driven MB. Modeling MYC-driven MB is critical for developing and testing potential therapies for this highly aggressive MB. Recently, murine MYC-driven MB models have been developed using mouse neural stem cells (NSCs) or neuronal precursor cells (NPCs). But human MB models derived from individual-specific cells are still lacking. Human induced pluripotent stem cells (iPSCs) can be differentiated into various types of cells and hold great promise for developing individual-specific disease models. It

is valuable to develop MB models using human iPSCs from both MB patients and unaffected persons. In comparison with mouse-cell-derived MB models, human-iPSC-derived MB models will provide a unique and high-impact platform not only for personalized drug discovery but also for studying the role of individual's distinctive genetic background in carcinogen sensitivity and MB susceptibility. Transcription factor Atoh1 governs the development of cerebellar granule neurons and is essential for MB formation. Here, we induced Atoh1 in human iPSCs to differentiate these cells into NPCs. We further infected these Atoh1-induced NPCs with lentiviruses encoding a stabilized form of MYC (MYCT58A) and dominant-negative p53 (DNp53). These NPCs generated aggressive tumors after being transplanted into mouse cerebellum. NPCs infected by DNp53 alone did not form tumors after 90 days. These MYC-driven tumors were comprised of poorly differentiated, medium to large size cells which showed nuclear molding, prominent nucleoli and numerous mitotic (Ki67+) and apoptotic (Cleaved-Caspase-3+) cells. These tumors also expressed early neuronal lineage marker (β -tubulin III). All these features closely mimic human Group 3 MB. Moreover, we also established neurosphere cultures from these MYC-driven tumors to enrich cancer stem-like cells that have the capability for long-term self-renewal and tumor initiation upon serial transplantations. In summary, we established a novel human-iPSC-driven cancer model for modeling MYC-driven MB. Our results support the feasibility to recapitulate human cancers using progenies derived from human iPSCs. The iPSC-derived MB model we established will facilitate mechanistic studies and drug testing for human aggressive MB.

American Association for Cancer Research

**615 Chestnut St. 17th Floor
Philadelphia, PA 19106**



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Tuesday, July 07, 2015

Re: AACR Annual Meeting 2015 in Philadelphia, PA

Abstract Control Number: 1672

Title: A MYC-driven medulloblastoma model derived from human induced pluripotent stem cells

Dear Dr. Ying:

Your above-referenced abstract has been scheduled for oral presentation in a Minisymposium session at the 2015 AACR Annual Meeting in Philadelphia, PA and will be published in the 2015 *Proceedings of the American Association for Cancer Research*. Presentation information pertaining to your abstract is below:

Session Category: Tumor Biology 1

Session Date and Time: Monday Apr 20, 2015 3:00 PM - 5:00 PM

Permanent Abstract Number: 2892

Please refer to the printed Final Program [distributed onsite] or the online Annual Meeting Itinerary Planner [available in late February through the AACR Website at <http://www.aacr.org>] for the exact location of your presentation.

[Instructions for Presenters in Minisymposia](#) are available through the 2015 AACR Annual Meeting website. Please visit the website for more information on oral presentations.

Minisymposium presenters at the AACR Annual Meeting must register for the full meeting at the rate appropriate to their membership status and obtain their own hotel accommodations. Registration and housing information are included below:

Advance Registration Deadline: February 9, 2015

[Online Registration](#) and Letters of Invitation

Special Offer for Non-Member Presenters

If you are not currently a member of the AACR, there is still time to join and enjoy significant savings

on your registration for the 2015 AACR Annual Meeting. Individuals who are interested in joining the AACR and registering at the advance member rate must submit a membership application no later than **5:00 PM ET Friday, February 6**. Interested candidates can apply for membership online at <http://myaacr.aacr.org/Default.aspx>. If you have any questions or need further information on becoming an AACR member, please contact the AACR Member Services Department at (215) 440-9300 or membership@aacr.org.

Housing Deadline: March 4, 2015

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**[Airline/Train Reservations; Ground Transportation;
Official Letters of Invitation for International Attendees]**

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Thank you for your participation in the 2015 AACR Annual Meeting.

Sincerely,
Lewis C. Cantley, Ph.D.
Program Committee Chairperson

PLEASE NOTE: This document is your official notice of acceptance. No separate letter of acceptance will be mailed.

Your Response: Confirm

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Status: Completed

WebcastSelectionNotification: Agree to participate including all slides

WebcastSignature: ying@kennedykrieger.org

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Mingyao Ying

eRA COMMONS USER NAME (credential, e.g., agency login): MINGYAOYING

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai, China	B.S.	07/2000	Microbiology
Fudan University, Shanghai, China	Ph.D.	08/2005	Developmental Biology
Johns Hopkins University, Baltimore, USA	Postdoc	08/2008	Parkinson's disease
Kennedy Krieger Institute, Baltimore, USA	Postdoc	11/2011	Stem cell biology

A. Personal Statement

I received my scientific training in the field of brain tumors and neurodegenerative diseases. My research focuses on studying the molecular mechanisms that regulate brain tumor stem cells and normal pluripotent stem cells.

I have published extensively on brain tumor stem cells. We found a novel transcription factor, kruppel-like factor 9 (KLF9), which can differentiate glioblastoma stem cells, inhibit their tumor propagation, and suppress Notch signaling in these cells (published in Stem Cells). We further defined genome-wide gene targets of KLF9, by using next-generation sequencing (published in JBC). We also identified retinoic acid as a differentiating agent that depletes glioblastoma stem cells by inhibiting Notch signaling (published in Oncogene).

After receiving my faculty appointment in 2011, I focus my research on identifying novel therapeutic targets for treating malignant brain tumors, including glioblastoma and medulloblastoma. Supported by the Discovery Grant from American Brain Tumor Association, We found for the first time that hyaluronan-mediated motility receptor maintains the self-renewal and tumorigenicity of glioblastoma stem cells, providing a novel therapeutic target for glioblastoma (published in Cancer Research). In 2014, I received the Career Development Award from US Department of Defense to apply human induced pluripotent stem cells to model high-risk MYC-driven medulloblastoma, and to further study downstream targets of MYC and Atoh1, two tumor-promoting transcription factors in medulloblastoma. We have established a novel stem-cell-derived model for MYC-driven medulloblastoma, and identified potential therapeutic targets for medulloblastoma. This work was selected for oral presentation during 2015 American Association for Cancer Research Annual Meeting.

Overall, I has a demonstrated record of successful and productive research in the field of brain tumors. My expertise and experience has prepared me to lead the proposed studies on AngII-induced MYC expression in medulloblastoma.

1. Jessica Tilghman, Hao Wu, Yingying Sang, Xiaohai Shi, Hugo Guerrero-Cazares, Alfredo Quinones-Hinojosa, Charles G. Eberhart, John Laterra and **Mingyao Ying**
HMMR maintains the stemness and tumorigenicity of glioblastoma stem-like cells.
Cancer Res. 2014 Jun 1;74(11):3168-79.

2. **Mingyao Ying**, Yingying Sang, Yunqing Li, Hugo Guerrero-Cazares, Alfredo Quinones-Hinojosa, Angelo L. Vescovi, Charles G. Eberhart, Shuli Xia, John Laterra.
KLF9, a differentiation-associated transcription factor, suppresses Notch1 signaling and inhibits glioblastoma-initiating stem cells.
Stem Cells. 2011 Jan; 29(1):20-31. [Highlighted on the cover]

3. **Ying M**, Tilghman J, Wei Y, Guerrero-Cazares H, Quinones-Hinojosa A, Ji H, Laterra J. KLF9 Inhibits Glioblastoma Stemness through Global Transcription Repression and Integrin- α 6 Inhibition. J Biol Chem. 2014 Nov 21;289(47):32742-56.
4. **Mingyao Ying***, Shervin Wang*, Yingying Sang, Peng Sun, C. Rory Goodwin, Alfredo Quinones-Hinojosa, Bachchu Lal, John Laterra and Shuli Xia. Regulation of glioblastoma stem cells by retinoic acid: role for Notch pathway inhibition. Oncogene. 2011 Aug 4;30(31):3454-67. (*: contributed equally)

B. Positions and Honors

Positions

2006-2008	Postdoctoral Fellow, PI: Ted Dawson, MD, PhD. Institute for Cell Engineering, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
2008-2011	Postdoctoral Fellow, PI: John Laterra, MD, PhD. Department of Neurology, Hugo W. Moser Research Institute at Kennedy Krieger and Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
2011-2012	Instructor Department of Neurology, Hugo W. Moser Research Institute at Kennedy Krieger and Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
2012-Current	Assistant Professor Department of Neurology, Hugo W. Moser Research Institute at Kennedy Krieger and Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Honors

2000	Scholarship for New Graduate Student, 1 st Prize, Fudan University
2009	Postdoctoral Fellowship Grant, Maryland Stem Cell Research Fund
2011	Scholar-in-Training Award, American Association for Cancer Research
2014	Career Development Award, US Department of Defense

C. Contribution to Science

1. Identifying potential therapeutic targets for depleting brain tumor stem cells.

My collaborators and I have made significant contribution to understanding the molecular mechanisms that regulates brain tumor stem cells, in particular glioblastoma stem cells (GSCs). We identified a number of potential therapeutic targets or strategies for depleting GSCs. We found that retinoic acid effectively differentiates GSCs and inhibits GSC tumorigenicity, by suppressing signaling pathways (e.g. Notch) essential for GSC self-renewal (published in Oncogene). For the first time, we identified KLF9, a differentiation-associated transcription factor, suppresses Notch1 signaling and inhibits GSC self-renewal and tumorigenicity (published in Stem Cells). By using next-generation sequencing, we further demonstrated that KLF9 Inhibits GSCs through global transcription repression and inhibiting Integrin- α 6, an essential regulator of GSC self-renewal (published in JBC). Most recently, we found for the first time that hyaluronan-mediated motility receptor (HMMR) maintains the self-renewal and tumorigenicity of GSCs, suggesting a novel therapeutic target for GBM (published in Cancer Research). I served as the primary investigator in all of these studies. These discoveries have provided novel mechanistic insights into the regulation of brain tumor stem cell and also provided a solid foundation for developing effective strategies for treating malignant brain tumors.

1. Jessica Tilghman, Hao Wu, Yingying Sang, Xiaohai Shi, Hugo Guerrero-Cazares, Alfredo Quinones-Hinojosa, Charles G. Eberhart, John Laterra and **Mingyao Ying** HMMR maintains the stemness and tumorigenicity of glioblastoma stem-like cells. Cancer Res. 2014 Jun 1;74(11):3168-79.

2. **Mingyao Ying***, Shervin Wang*, Yingying Sang, Peng Sun, C. Rory Goodwin, Alfredo Quinones-Hinojosa, Bachchu Lal, John Laterra and Shuli Xia.

Regulation of glioblastoma stem cells by retinoic acid: role for Notch pathway inhibition.

Oncogene. 2011 Aug 4;30(31):3454-67. (*: contributed equally)

3. **Mingyao Ying**, Yingying Sang, Yunqing Li, Hugo Guerrero-Cazares, Alfredo Quinones-Hinojosa, Angelo L. Vescovi, Charles G. Eberhart, Shuli Xia, John Laterra.

KLF9, a differentiation-associated transcription factor, suppresses Notch1 signaling and inhibits glioblastoma-initiating stem cells.

Stem Cells. 2011 Jan; 29(1):20-31. [Highlighted on the cover]

4. **Ying M**, Tilghman J, Wei Y, Guerrero-Cazares H, Quinones-Hinojosa A, Ji H, Laterra J.

KLF9 Inhibits Glioblastoma Stemness through Global Transcription Repression and Integrin- α 6 Inhibition.

J Biol Chem. 2014 Nov 21;289(47):32742-56.

2. Developing highly efficient strategies for differentiating human pluripotent stem cells.

I have been applying my knowledge and methodology from cancer stem cell research for studying human pluripotent stem cells. We focus on how to efficiently differentiate human induced pluripotent stem cells into lineage-specific progenies, such as dopaminergic neurons, for disease modeling and cell replacement therapy. We have established a highly efficient differentiation method for deriving midbrain dopaminergic neurons from human induced pluripotent stem cells (published in Stem Cells Translational Medicine and patent pending). I served as the primary investigator in this study. Recently, we further applied this differentiation method to generate a MYC-driven medulloblastoma model derived from human induced pluripotent stem cells, which study provided a unique and high-impact platform for developing personalized therapies and studying the role of an individual's genetic background in medulloblastoma susceptibility.

1. Jonathan Sagal, Xiping Zhan, Jinchong Xu, Jessica Tilghman, Senthilkumar S. Karuppagounder, Li Chen, Valina L. Dawson, Ted M. Dawson, John Laterra and **Mingyao Ying**

Proneural Transcription Factor Atoh1 Drives Highly Efficient Differentiation of Human Pluripotent Stem Cells into Dopaminergic Neurons.

Stem Cells Transl Med. 2014 Aug;3(8):888-98.

2. **Patent: Mingyao Ying** and John Laterra. Method for highly efficient conversion of human stem cells to lineage-specific neurons. PCT/US14/68273, Receipt date: 03-DEC-2014.

3. Establishing models for neurodegenerative diseases and studying underlying molecular mechanisms.

My early publications provided novel animal models for studying neurodegenerative diseases, such as Parkinson's disease and polyglutamine expansion diseases. We established the first conditional transgenic mice that express mutant human alpha-synuclein found in Parkinson's disease patients. These mice serve as novel animal models for studying striatal dopamine reduction in Parkinson's disease. We found for the first time that the transcription factor Atrophin-1 with polyglutamine expansion induces transcriptional deregulation and neurodegenerative phenotypes in a mouse model of Dentatorubral-pallidoluysian atrophy. We further established an effective therapeutic strategy for this mouse model by using the histone deacetylase inhibitor sodium butyrate. I served as the primary investigator in these studies.

1. Daher JP*, **Ying M***, Banerjee R, McDonald RS, Hahn MD, Yang L, Flint Beal M, Thomas B, Dawson VL, Dawson TM, Moore DJ.

Conditional transgenic mice expressing C-terminally truncated human alpha-synuclein (alphaSyn119) exhibit reduced striatal dopamine without loss of nigrostriatal pathway dopaminergic neurons.

Molecular Neurodegeneration. 2009 Jul 24;4:34. (*: contributed equally)

2. **Ying M**, Xu R, Wu X, Zhu H, Zhuang Y, Han M, Xu T.

Sodium butyrate ameliorates histone hypoacetylation and neurodegenerative phenotypes in a mouse model for DRPLA.

J Biol Chem. 2006 May 5;281(18):12580-6.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/mingyao.ying.1/bibliography/46816514/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

Maryland Stem Cell Research Fund 7/1/15—6/30/17

Exploratory Research Grant (RFA-MD-15-2)

Highly Efficient Conversion of Human iPS Cells to Dopaminergic Neurons by Synthetic Modified mRNAs.

We propose to develop a synthetic-mRNA-based differentiation strategy for efficiently generating functional and transplantable dopaminergic neurons from human pluripotent stem cells.

Role: Principle-Investigator

Department of Defense Congressionally Directed Medical Research Programs (CDMRP) 7/1/2016 - 6/30/2017
Career Development Award (CA130319) (no-cost extension)

Modeling Aggressive Medulloblastoma Using Human-Induced Pluripotent Stem Cells

We propose to study: Aim 1: To establish a human-iPSC-derived MYC-driven medulloblastoma (MB) model and test the effects of Atoh1 expression on tumor initiation and growth in this MB model; Aim 2: To identify the genome-wide MYC-regulated transcriptional network responsible for MYC-driven tumorigenesis, and to determine the effects of Atoh1 expression on MYC-regulated transcriptional events.

Role: Principle-Investigator

There is no budgetary or scientific overlap between the ongoing grants and current application.

Completed Research Support

Maryland Stem Cell Research Fund 7/1/12—6/30/14

Exploratory Research Grant (2012-MSCRF-0198-00)

Highly efficient conversion of human stem cells to dopaminergic neurons by proneural transcription factor Atoh1

We propose to develop a transcription-factor-based differentiation strategy for efficiently generating functional and transplantable dopaminergic neurons from human pluripotent stem cells.

Role: Principle-Investigator

American Brain Tumor Association 7/1/12 — 6/30/13

Discovery Grant

Hyaluronan-mediated Motility Receptor as a Novel Target for Inhibiting Glioblastoma Stem Cells

In these proposed studies, we expect to identify HMMR as a novel therapeutic target against glioblastoma multiforme (GBM) by efficiently killing GBM stem cells. These studies will lay an essential foundation for the development of HMMR-targeting therapy, such as HMMR inhibitors or monoclonal antibodies, as new anti-GBM therapy.

Role: Principle-Investigator

Maryland Stem Cell Research Fund 07/01/2009 -- 06/30/2011

Postdoctoral fellowship

Differentiation Therapy Induced by Kruppel-like Factor in Glioblastoma Stem cells.

The goal of this project is to study the role of kruppel-like transcription factor 9 in glioblastoma stem cells.

Role: Principle-Investigator

National Institute of Health 9/15/11 – 7/31/16

1R01NS076759-01

Suppression of Glioblastoma Stem Cells by Kruppel-Like Factor 9

The research focuses on understanding glioblastoma stem cell regulation by the KLF9 transcription factor and to develop KLF9-based Brain cancer treatment strategies.

Role: Co-Investigator; Principle-Investigator: John Laterra

Maryland Stem Cell Research Fund 6/1/11 – 6/30/14

2011-MSCRFII-0073-00

Regulation of Neural and Neoplastic Stem Cells by Kruppel-like Transcription Factors

This research focuses on understanding how multiple KLF transcription factors differentially regulate neoplastic and non-neoplastic neural stem cell biology and gene expression.

Role: Co-Investigator; Principle-Investigator: John Laterra

Maryland Stem Cell Research Fund

7/1/12—6/30/14

Exploratory Research Grant

Global Prediction of Transcription Factor Binding Sites in Lineage Specific Neural Differentiation

This project will develop the first “Hi-3” approach for predicting transcription factor binding sites of 500+ transcription factors in many biological contexts simultaneously. We will apply the new Hi-3 approach to study neuronal cell-fate determining regulatory networks during human neural progenitor cell differentiation.

Role: Co-Investigator; Principle-Investigator: Hongkai Ji